NITROGEN TRANSFORMATION PRODUCTS ELIMINATE PLANT PATHOGENS IN SOIL

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Previous reports to the MBAO conference by our laboratory demonstrated that various N-amendments (meat and bone (MBM), feather and blood meals) reduced the incidence of soilborne diseases of soils cropped to potatoes. The products were effective in reducing the incidence of early dying syndrome (caused by *Verticillium dahliae*), common scab (*Streptomyces scabies*), and lesion, pin, and rootknot nematodes (various genera) (Lazarovits *et al. in press*). These products when applied to soil have the potential as an alternative to methyl bromide (MB). At present, use of N-amendments as a disease control strategy is limited by; a) a requirement for uneconomically high application rates, b) potential for excessive nitrogen application to soil and c) inconsistent control of pathogens from field to field. The objective of this research was to investigate the mechanisms by which N-amendments kill soil pathogens so that the effective rate may be reduced. To that objective we also examined what soil properties make the products more effective. Such information is vital to advise growers in using N-amendments as an alternative to MB.

The fungus *Verticillium dahliae* (Kleb.), a wilt pathogen of many crops, was used as a model pathogen in these studies. In potato (*Solanum tuberosum*), *Verticillium* wilt causes premature senescence and the disease referred to as “early dying syndrome”. Infection of potato plants occurs when roots contact microsclerotia (MS) of *V. dahliae*. Microsclerotia which consist of clustered, melanized, thick-walled and hyaline, thin-walled hyphal cells can survive in soil for many years and are only readily eliminated from soil by fumigation.

Model System: The viability of *V. dahliae* MS retrieved from soil was determined as follows. Desired levels of MBM were mixed with soil and 20 g of the mixture added to test tubes. Microsclerotia, mixed with crushed silica sand, were added to a nylon pouches and buried in soil or suspended in the headspace of each tube. Water was added to each tube to bring soil moisture to 0.33 bar tension, the tubes were loosely capped, and placed in the dark at 24^{o}C. On each date of analysis, nylon pouches were retrieved and an Anderson soil sampler used to impact the contents by vacuum onto medium selective for growth of *V. dahliae*. Viability of MS were determined two weeks following plating. The numbers germinated from a total of 50 MS were recorded. The mortality of MS determined using this bioassay has been shown to relate well to reduction in *Verticillium* disease incidence in greenhouse and field grown potatoes (Lazarovits *et al. in press*).

Soil pH and levels of NH_3, NO_2^- and NO_3^- were determined on each sample date. Soil (8g) was mixed with cold distilled water (40mL) in sealed plastic bags, the slurry mechanically disrupted with a laboratory blender and shaken at 5^oC for 1 h. The slurry once again mechanically disrupted and the pH of the slurry determined and the slurry centrifuged. The supernatant was then analyzed for total NH_3+NH_4^+, HNO_2+NO_2^- and NO_3^- using an ion chromatograph. Ammonia (NH_3) and nitrous acid (HNO_2) were calculated as the fraction of total NH_3+NH_4^+ or NO_2^-+HNO_2 respectively in solution using the Henderson-Hasselbalch equation knowing soil pH and incubation temperature.

Mechanism Studies: N-amendments found to kill MS placed in or suspended above soil, included MBM, soymeal, bloodmeal, feathermeal, poultry manure, urea, NH_3, $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3 and $NaNO_2$. In some instances the kill of MS occurred in less than a week but in other cases, the MS died well after 2 weeks of amendment. The rapid kill was generally associated with high soil pH values (> 8.5) whereas, the slower kill occurred after a decline in soil pH (< 6).

The cycling of nitrogenous compounds was obviously involved in MS mortality. One of the approaches to test this was to inhibit nitrification in amended soil with the inhibitor, dicyandiamide (DCD). Various rates of MBM were added to a loamy sand with or without DCD. With 2% (w w^{-1}) MBM, MS were killed within 10 days after amendment regardless of addition of DCD (Fig. 1). This early kill of MS corresponded with NH_3 levels in soil of around 20m*M* (280ppm-N). With the1% rate of MBM, MS were killed after 2 weeks but only in soil without DCD. This delayed kill of MS was associated with the accumulation of HNO_2 levels above 0.02 m*M* (0.28ppm-N).

If HNO_2 accumulation during nitrification is required for the delayed kill of MS, then the form of N-fertilizer applied will influence MS survival. Accordingly various N-fertilizers were added to soil (to about 400 and 800 kg N ha^{-1}) and MS survival and HNO_2 accumulation estimated. All sources of N-fertilizer capable of being converted to nitrate (urea, $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3) or yielding HNO_2 directly ($NaNO_2$) were found to kill MS (Fig. 2). The kill of MS corresponded with HNO_2 levels above 0.02 m*M* in soil solution. The nitrate form of fertilizer that did not yield any HNO2 had no effect on MS survival.

The observation that N-amendments that undergo nitrification also affect the mortality of MS was confirmed in field microplots. MBM, $(NH_4)_2SO_4$ and KNO_3 were added to about 1000 kg N ha^{-1} and MS survival and HNO_2 accumulation estimated MS were killed 4 or 6 weeks after amendment with the MBM and $(NH_4)_2SO_4$ treatments, but survived with KNO_3 addition (Fig. 3). Death of MS again corresponded to HNO_2 accumulation in soil.

Thus evidence is provided for two mechanisms of killing MS in N-amended soil; one being NH_3, the other HNO_2 toxicity. Both compounds, tested in solution or in atmosphere, were found to be lethal to MS at levels estimated in amended soil. NH_3 and HNO_2 at levels found in N-amended soils was also shown to be toxic to a

number of other soilborne pathogens tested including spores of *Streptomyces scabies*, chlamydospores of *Fusarium oxysporum* f. *lycopersici*, and sclerotia of *Sclerotinia sclerotiorum* (data not presented). Thus, both mechanisms have a broad spectrum of control of soilborne plant pathogens.

Soil pH is the overriding factor determining kill of MS. Measuring soil pH can provide a fast means to predict which mechanism may be active (Fig. 4). For NH_3 toxicity, the pH must increase above 8.5 because only at these values is the non-toxic NH_4^+ converted to toxic NH_3. Ammonification of organic N or hydrolysis of urea is responsible for driving pH up following amendment. Soil organic matter seems to control the pH rise and NH_3 accumulation in soil. Soil with > 3% organic matter cannot accumulate sufficient NH_3 to kill MS when amended to 2% MBM. Higher rates of MBM are necessary in these soils to kill MS by this mechanism. Studies are in progress to determine how organic matter prevents NH_3 accumulation and toxicity in soil.

A soil pH below 5.5 is necessary to induce HNO_2 toxicity. At these pH values, NO_2^- accumulates under conditions of rapid nitrification of N-amendments added to soil. Nitrification is an acidifying process that reduces pH thus converting non-toxic NO_2^- to toxic HNO_2 (Fig. 4.). The ability of a soil to buffer against acidity determines if soil pH is lowered sufficiently to induce HNO_2 toxicity. We have developed a simple titration assay in which various amounts of H_2SO_4 is added to soil and pH determined. Soils without carbonates have poor acid buffering capacity. It is in these soils, that we have found HNO_2 accumulation and kill of MS.

<u>Conclusions:</u> N-amendments applied to soil can be an effective broad spectrum and practical alternative to MB for some soils (Table 1). However, if these products are to be used as an alternative to MB, the type and rate of N-amendment must be matched to the soil properties of an individual field.

<u>Note:</u> This study was conducted by the first author towards partial fulfillment of a doctoral degree. Dissertation and manuscripts are in preparation. Funding for this research was provided by the Fats and Proteins Foundation of the United States of America.

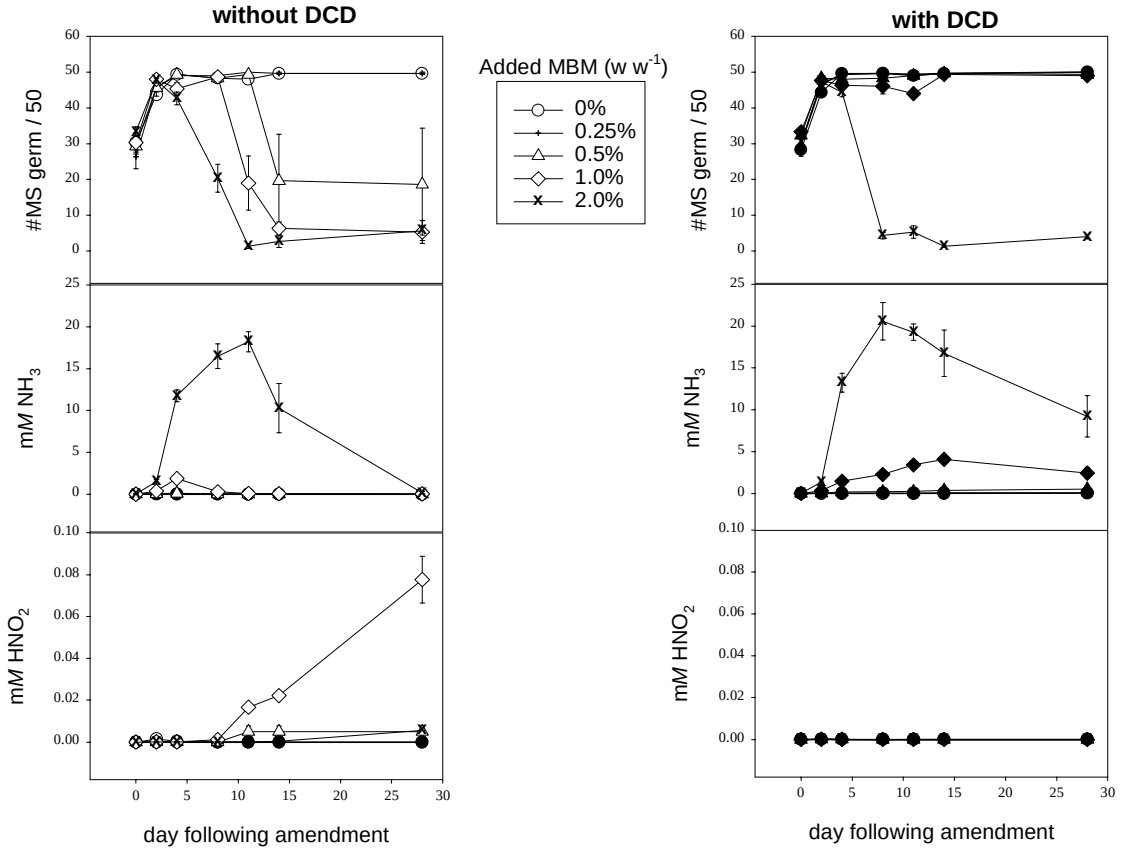

Fig. 1. MS germination, NH_3 & HNO_2 concentration, in a soil with low organic matter and poor acid buffering capacity amended with MBM and ± DCD added (n=3; ±S.E.).

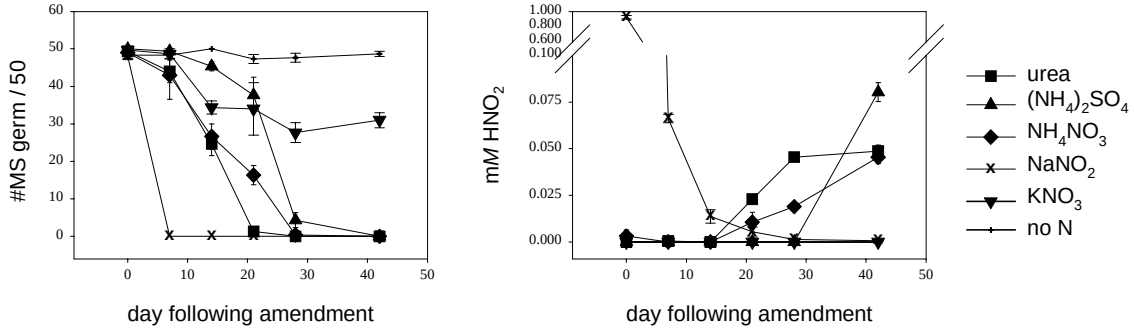

Fig. 2. MS germination and HNO_2 concentration in a soil with low organic matter and poor acid buffering capacity amended with different fertilizers (800 kg N ha^{-1}, n=3; ±S.E.).

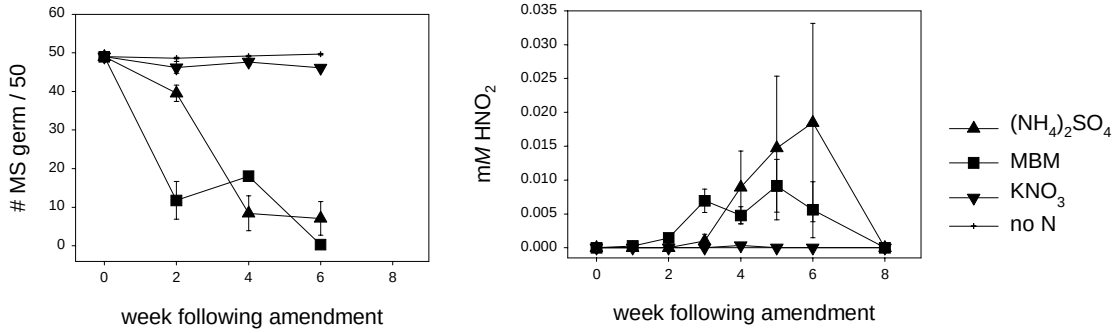

Fig. 3. MS germination and HNO_2 in soil with low organic matter and moderate acid buffering amended with various N sources (1000 kg N ha^{-1}) in microplots (n=3; ±S.E.).

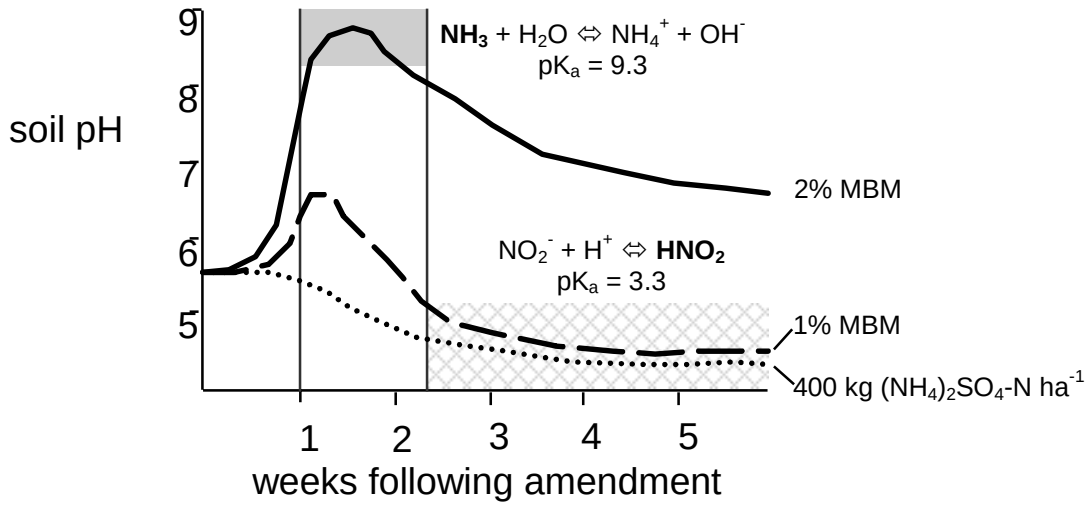

Fig. 4. Typical changes in soil pH following incorporation of various N amendments in a soil with low organic matter content and poor acid buffering capacity. pH values above 8.5 are required for presence of ammonia (NH_3) toxicity (grey zone). For nitrous acid (HNO_2) toxicity, pH values below 5.5 are required (cross-hatched zone).

Table 1. Summary of mechanisms envolved in the control of *Verticillium dahliae* in soil by the addition of N amendments.

	NH_3 Mechanism	**HNO_2 Mechanism**
Min. Lethal Conc. (24 h)	>170 ppm(N) in solution	>2 ppm(N) in solution
Location	soil solution or atmosphere	soil solution or gas
Type of Amendment	Org-N products (>8% N), urea, anhydrous NH3	Org-N products, fert-N (not NO_3^-)
Rate of Application	>1600 kg N ha^{-1}or >20 tons ha^{-1} org-N product	>400 kg N ha^{-1} or >20 kg NO_2^- -N ha^{-1}
Determining Soil Properties	organic matter	pH <6.0, poor acid buffering ability, rapid nitrification
Time After Amendment	4-14 days	2-6 weeks
Phytotoxicity	planting delayed 1-2 months	not evident